



The cypermethrin toxicity effect of *Poecilia latipinna* histological changes

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Abstract

The effect of cypermethrin insecticide in an aquarium fish *Poecilia latipinna* commonly known as black molly was studied. The samples of 150 fishes were collected from Kolathur of Chennai. Behavioral responses were observed in the control group of fishes which exhibits normal swimming activity and normal opercula movements. Fishes exposed to 0.2, 0.4, 0.6, 0.8 and 1 ppm of cypermethrin noticed many changes in the behavioral response loss of balancing, increased opercula beats, erratic swimming, swimming at the water surface and gulping for air noticed in the experimental fish. The 96 hr LC₅₀ value of the fish exposed to various concentrations of cypermethrin ranging from 0.2 to 1 ppm was plotted and the LC₅₀ values found to be 0.7 ppm. The histopathological changes observed in the liver of *Poecilia latipinna* exposed to 0.175 ppm concentration at 24 hours, 96 hours, 240 hours and 360 hours includes organization of the hepatic cell, completely vacuolated nuclei, more number of vacuolation in hepatocytes, decrease in size of nucleus (Pycnotic). The histomorphology of the intestine in the control fish exhibited simple columnar epithelium, lamina propria, sub mucosa, tunica muscularis and serosa. A few goblet cells containing lymphocytes were located between the epithelial cells. The mucosa was highly branched and folded. The intestine of the experimental fish revealed damage to the appearance of few goblet cells, disintegration of cell wall, and shrinkage of columnar epithelial cells, Damage of columnar epithelial cells and hyperactivity and appearance of more number of goblet cells. The histopathological changes of the liver and intestine were studied in control and experimental fishes using Haematoxylin and Eosin based on the observation, the effect of cypermethrin was discussed with light of the available literature.

Keywords: histopathological, intestine, Behavioral responses, *Poecilia latipinna*, cypermethrin

Introduction

Fish are particularly sensitive to a wide variety of pesticide chemicals and toxic concentration may rise not only from spillage of agricultural practical if their use is excessive but also from several other sources. The industrial development and rapid urbanization has led to development of polluted zones discharging potentially toxic compounds in the environment toxicology studies followed (V Tamizhazhagan & Pugazhendy, 2015^[13]; V Tamizhazhagan, Pugazhendy, Sakthidasan, & Jayanthi, 2016^[16]; Usha, Pugazhendy, Tamizhazhagan, Sakthidasan, & Jayanthi, 2017^[18, 21-27]; Vijayan, Senthilmurugan, Pugazhendy, & Tamizhazhagan, 2018) and Padmapriya *et al.*, 2017a.^[21, 23], Usha *et al.*, 2017b.^[18, 22], Pitchaimani *et al.*, 2017a., Jayakumar *et al.*, 2018)^[28]. Apart from causing death either directly or due to starvation by destruction of food organisms many pesticides have been shown to effect growth rate, reproduction and behavior with the evidence of tissue damage (Tilak *et al.*, 2005)^[17]. Owing to the excessive use of synthetic pyrethroids, the environment and water resources are being polluted, thus endangering aquatic life directly and human life indirectly (Hill, 1989)^[8]. The bivalves exposed to several environmental factors may reflect adaptive metabolic mechanisms due to the challenges of the changing environment including the pollutants (Savir & Patil, 1994)^[10]. Domestic sewages are run off from agriculture fields loaded with pesticides and fertilizers, pollute the water bodies. Commonly used pesticides can be harmful

living organisms, pets, and their environment (V Tamizhazhagan & Pugazhendy, 2016b)^[15] Bioassay of heavy metals in marine and coastal ecosystem is of great concern for decline or loss of fishery resources since past several decades (Firestone, 1982; Waldichuk, 1974)^[5, 20] and (Eister, 1978)^[4]. Heavy contamination of pesticides in water in turn leads to oxygen depletion and cases of poisoning and the mass mortality of fishes is not uncommon.

The recently introduced synthetic pyrethroids with multiple beneficiary qualities have attracted farmers to use these compounds in pest control. But these compounds have been found to be highly toxic to fish (Dhawan & Kaur, 1996)^[2]. Pesticides are generally used in contemporary agriculture to aid in the manufacture of high quality food (Vairakkannu Tamizhazhagan, 2017)^[12, 18, 21-27]. A number of chemicals released into environment contaminate especially aquatic ecosystem through different ways (Usha *et al.*, 2017)^[18, 22]. Domestic sewages are sprint decomposed commencement agriculture field weighed down with pesticides and fertilizers contaminate the water body tissues or organisms at very low levels (Vairakkannu Tamizhazhagan, 2017)^[12, 18, 21-27]. Pesticides represent some of the most spread pollutants, raising difficult problems for the environmental production as the cause noxious effect upon certain useful organisms. The use of pesticides has increased with the growing awareness about their utility in agriculture production, animal husbandry and post harvest technology and in the public health and

welfare of mankind. Fish is very sensitive to changes in water due to addition of effluents and toxicants (Djuniadi, 2005; Ghosh, Mukherjee, Pal, & Mondal, 2014) ^[3, 6] and changes occurring in the biochemical characteristics of fish provide a sensitive measure to know the health of fish fauna (Graetz & Nair, 2009) ^[7] Most heavy metals are released into the aquatic phase as a result of direct input, atmospheric deposition and erosion due to rain water and therefore aquatic animals may be exposed to elevated levels of heavy metals due to their wide use of anthropogenic purpose (Aruldoss, Indra, & Sankar Samipillai)

The introduction of small amounts of many relative toxic materials in an aquatic environment cause multiple change in the internal dynamics of aquatic organism at sublethal levels: in aquatic toxicology, extensive literature is available on effects of various pollutants on the biochemical composition of tissue of different types in marine bivalve (Shirwaikar, Shirwaikar, Rajendran, & Punitha, 2006) ^[11] from the available literature it appears that the study of biochemical composition is very useful in understanding the mechanism of metal toxicity to green mussels. Food is an important source of energy for all living organisms. Food energy is used for building up body tissue, which further signifies that a balanced diet is necessary for proper functioning of the body. Recent understanding of different biochemical processes has proved useful in determining the mechanism of toxicity in different toxicants also in unfolding the adaptive protective mechanisms of the body to combat the toxic effect of the pollutants, besides it is also observed that some biochemical alteration occurring in the body give the first indication of stress in the organism and hence the efforts, on the part of pollution biologists to explore the possibility of making use of the phenomenon to locate certain type of pollutants in nature. In the present study an attempt has been made to observe the histopathological changes induced by a synthetic pyrethroid, cypermethrin on histology, of certain vital organs namely intestine and liver of an aquarium fish *Poecilia latipinna* commonly known as black molly.

Material and Methods

Collection and maintenance of fishes

Live specimen of *Poecilia latipinna* commonly known as black molly is used for present study. A total number of 150 fishes procured from Kolathur of Chennai and transported to the laboratory with sealed polythene bags filled with oxygen. The size of the fish was ranged between 5 cm and 7 cm in length. The fishes acclimatized in the laboratory for about 15 days. They were fed with formulated fish feed and boiled egg and the water was exchanged every alternative days.



Fig 1: Experimental animal

Determination of lethal concentration (LC₅₀)

Stock solution of Cypermethrin (10% EC) (Sicorin 10 Scientific fertilizer Co. Pvt., Ltd., Trichy) pesticide was prepared by diluting 1ml insecticide in 100ml of distilled water. From the stock solution different concentrations of Cypermethrin, 0.2, 0.4, 0.6, 0.8 and 1ppm were prepared by diluting the respective milliliter of the stock solution in one liter of test water. Healthy fishes (n=10) with an average weight of 2 gram and an average length of 5cm were maintained in each test solution for 4 days to determine the LC₅₀ values. Feeding was stopped one day prior to the experiment and also during the experimental period. The LC₅₀ values were found by arithmetic graph method. Graph was used in this type of analysis with percent survival on the ordinate against the concentration on the abscissa. Each data point is plotted and connected to form a graph. A horizontal line was drawn from the 50 percent survival point to intersect the plot. A vertical line from the intersection point was then dropped to the abscissa. This intersection point was then dropped to the abscissa. This intersection point on the abscissa corresponds to the 96 hours LC₅₀ (Reish & Oshida, 1987) ^[9].

Histological Techniques

Both the control and experimental fishes were dissected out liver and intestine tissues were removed from both the sets of fishes. The tissues were fixed in Bouin's fluid. The fixed tissues were processed using alcohol dehydration and Tetrahydrofurine for clearing. They were embedded in paraffin wax (congealing point 58-60° C) and longitudinal sections and transverse (6µm) were taken was followed by standard methods (Vairakannu Tamizhazhagan and Kannaiyan Pugazhendy, 2017 (Vairakkannu Tamizhazhagan, 2017) ^[12, 18, 21, 27].

Results

Observations on the behavioral responses of *Poecilia latipinna*, exposed to cypermethrin at different concentrations exhibited several pathological symptoms. The control group showed normal behavior during the test period. The loss of balancing, increased opercula beats, erratic swimming, swimming at the water surface and gulping for air noticed with the experimental fish of the present study. In 1 ppm concentrations, loss of balancing, frequent surfacing and rapid opercula movements were noticed. In 0.8 ppm concentrations, fishes were resting at bottom in crowds while in 0.6 ppm concentration, there was a reduction in swimming activity followed by mucous secretion from mouth and gills. In 0.4 ppm concentration, gills become pale and fishes were violent showing erratic swimming movements.

In contrast, the controls did not show any change in the behavioural symptoms with respect to swimming activity, opercula movements and mucous secretion. Controls responded very well to external stimulus. The LC₅₀ value for 96 hours duration of *Poecilia latipinna* exposed to cypermethrin was found to be 0.7 ppm. The arithmetic graph plotted for LC₅₀ value is presented in (Figure 1). The liver of the normal fish comprises a continuous mass of hepatic cells with cord like formation. Hepatic cells are roundish, polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structure known as

hepatic cell cords. Bile canaliculated is centrally located in each cord. A large number of blood sinusoids are found in the hepatic mass of these cords. Thin bile canaliculi are observed between the hepatic cells. Hepatic cells have many vital functions other than the secreting of bile. The observation of the experimental fish exposed to 0.175 ppm of cypermethrin at 24 hours shows less affected structure with hepatocytes showing prominent nuclei. Fish exposed to 96 hours duration with same concentration of cypermethrin showed enlargement of hepatic cells (EHC) and formation of vacuoles (FV). The fish exposed to cypermethrin at 0.175 ppm concentration with 240 hours, noticed damages in the hepatic cells, pycnotic nucleus (PC) and necrosis of hepatocytes (NH).

The histopathological changes noticed in the liver of the exposed fish at the same concentration with the 360 hours exposure showing degeneration of cytoplasm in hepatocytes

(DCH) and disintegration of hepatocytes (DNH). The intestine of *Poecilia latipinna* exhibit the simple columnar epithelium, lamina propria, submucosa, tunica muscularis and serosa. The long thin columnar epithelial cells of the mucosa showing surface microvilli. A few goblet cells containing lymphocytes were located between the epithelial cells. The mucosa was highly branched and folded.

The observation of the experimental fish exposed to 0.175 ppm of cypermethrin for 24 hours showed with a few numbers of goblet cells. Intestine of the exposed to 0.6 ppm at 4 days duration shows more goblet cells. The sublethal exposure of cypermethrin of 0.6 ppm at 10 days duration showed more goblet cells and disintegration of cell wall. The intestine of the fish exposed to 0.6 ppm concentration at 15 days duration shows appearance of more goblet cells, slight shrinkage of columnar epithelial cells.

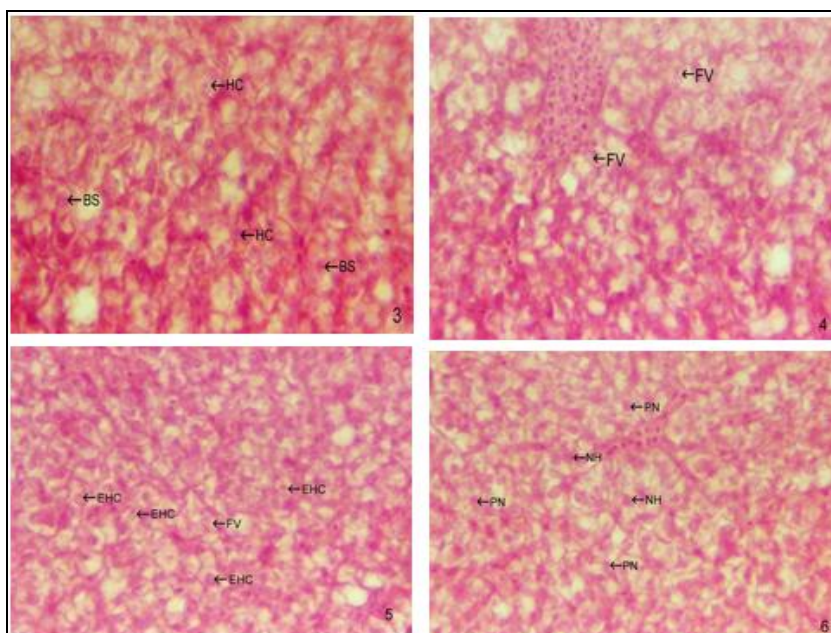


Plate 1: Figure Histopathological alteration in Liver tissues

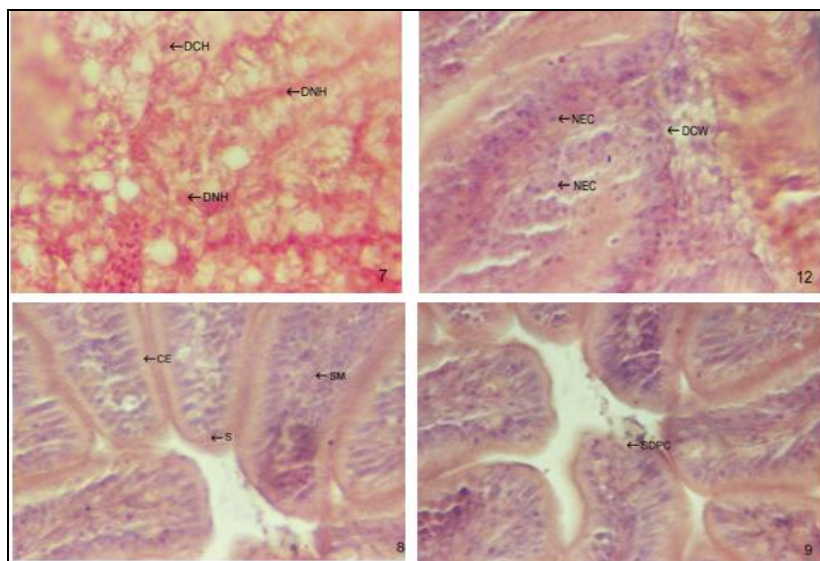


Plate 2: Histopathological changes in Intestine tissues

Discussion

The Observations pertaining to behavioral responses of *Poecilia latipinna*, exposed to cypermethrin at different concentrations exhibited several pathological symptoms in physiochemical alteration (V Tamizhazhagan & Pugazhendy, 2016a) ^[14]. The control group showed normal behavior during the test period. The loss of balancing, increased opercula beats, erratic swimming, swimming at the water surface and gulping for air noticed with the experimental fish of the present study. Fishes exposed to different pesticides and their behavioural changes in terms of toxic effect was reported by many workers (Ansari and Kumar, 1986; Mani and Konar, 1985 and Sumithra 2004). Similar responses to those of *L. thermalis* such as irregular swimming, abnormal opercular movement, gulping of air and frequent surfacing were reported upon caldan insecticide exposed to *Poecilia reticulata* (Sumithra, 2004).

The observation of the experimental fish exposed to 0.175 ppm of cypermethrin at 24 hours shows less affected structure with hepatocytes showing prominent nuclei. Fish exposed to 96 hours duration with same concentration of cypermethrin showed enlargement of hepatic cells (EHC) and formation of vacuoles (FV). The fish exposed to cypermethrin at 0.175 ppm concentration with 240 hours, noticed damages in the hepatic cells, pycnotic nucleus (PC) and necrosis of hepatocytes (NH). The histopathological changes noticed in the liver of the exposed fish at the same concentration with the 360 hours exposure showing degeneration of cytoplasm in hepatocytes (DCH) and disintegration of hepatocytes (DNH).

Tilak *et al.*, (2001) reported in *Ctenopharyngodon idellus* exposed to fenevalerate has induced discrete pathological changes in the liver tissues includes degeneration of cytoplasm in hepatocytes, atrophy formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords, degeneration in hepatocyte wall and decrease in size of nucleus (Pycnotic) observed. Porkodi (2005) reported similar changes noticed in the present study when *Gambusia affinis* exposed to a synthetic pyrethroid.

In the present investigation the fish exposed (V Tamizhazhagan & Pugazhendy, 2015 ^[13]; V Tamizhazhagan *et al.*, 2016; Usha *et al.*, 2017; Vijayan *et al.*, 2018) ^[21-27, 12, 18, 22, 19] to cypermethrin at 0.016 ppm concentration showed extensive histopathological changes in the liver includes damages of the hepatocytes, like vacuolation at 10 days duration, degeneration of cytoplasm in hepatocytes, formation of more vacuoles, disintegration of hepatocytes and decrease in size of nucleus (Pycnotic) observed, when fishes exposed to longer duration upto 30 days. All these histopathological changes also reported by Tilak *et al.*, (2001) and Anita Susan and Tilak (2003) as elucidated above.

The observation of the experimental Fish exposed to 0.175 ppm of cypermethrin for 24 hours showed with a few numbers of goblet cells. Intestine of the exposed to 0.6 ppm at 4 days duration shows more goblet cells. The sublethal exposure of cypermethrin of 0.6 ppm at 10 days duration showed more goblet cells and disintegration of cell wall. Sumithra (2004) reported the effect of caldan on the histology of intestine in *Poecilia reticulata*. The changes include shrinkage of lamina propria, vacuolation of columnar epithelial cells, damage and

disintegration of epithelial cells and goblet cells. Mandal and Kulshreshthra (1979) while studying the effect of Sumithion in the intestine of *clarius batrachus*, noticed lesion formation in villi, enlarged mucous cells, vacuolization of epithelial cells of mucosa and occurrence of cellular exudates in lumen.

Anitha and Kumar (1997) and Padmapriya *et al.*, 2017b., Usha *et al.*, 2017a., Pitchaimani *et al.*, 2017b., Jayakumar *et al.*, 2018) ^[18, 22, 21, 23, 29] have reported the histopathological changes in intestine due to the pollutants. The changes observed were degenerative villi, epithelial cells were degenerated and formed a syncytial mass. Mucous secreting goblet cells showed hyperactivity. Also seen were the oedematous epithelia and connective tissues, dilated blood vessels and lymphocyte migration. Plaha and Sahai (1989) noticed changes in *Heteropneustes fossilis* after gradual exposure in three pesticides noticed detachment of serosa, necrosis in both longitudinal and circular muscle layers, shrinkage of villi and ruptured walls and dilation of blood vessels. Wagh and Khillare (1989) explained the effects of combination of three pesticides viz., endosulfan, malathion and sevin. Among the three pesticides endosulfan toxicity was more severe as it caused degeneration of the villi besides damage of the epithelial cells, Malathion also showed insignificant damages in the fish *Barbus stigma*. As reported by Anitha and Kumar (1997) when fishes exposed to pollutants observed hyperactivity of goblet cells also noticed in the present study as *P. reticulata* exposed to cypermethrin at 0.007ppm concentration for longer duration like 192 hrs noticed more number of goblet cells with hyperactivity.

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